Embryo Grade I is The Most Important ICSI Parameters That Predict Intracytoplasmic Sperm Injection(ICSI) Outcome

Ban J. Edan* Sami R.Al-Katib** Basima Sh.AlGhazali**
*College of Medicine , Babylon University
**College of Medicine , Kufa University

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Abstract

The aim of this study was to compare different intracytoplasmic sperm injection (ICSI) parameters with its outcome and predict the most important factor that relate with successful rate.

A total seventy-five infertile women aged between 22-45 years(31.43 ± 5.38 years), referred to the fertility clinic in Al-Sadder teaching hospital, , and undergone intracytoplasmic sperm injection throughout period from March 2013 to January 2014,were included in this study. Fertilization rate (FR) and cleavage rate was calculated. The embryos grading was evaluated according to their morphology and percentage of fragmentation. The biochemical pregnancies was confirm on the fourteenth day of embryo transfer by measurement of serum human chorionic gonadotrophine (B- HCG).

Out of the 75 included patients, 15 (20%) achieved pregnancy after ICSI. Pregnant women were associated ($P<0.05$) . Total oocyte retrieved , stage metaphase II oocyte , pronucleus , cleavage rate , grade I ,total number of embryos ,and number of transferred embryo significantly higher in pregnant women when compared with non-pregnant women($P<0.05$) Analysis of ROC curve revealed that area under the curve for number of grade I embryos was 0.868 for predicting pregnancy followed by followed by MII (0.815), number of total oocyte .retrieved and PN (0.773), ET(0.767) and total number of embryos(0.758) respectively.

Data show that the number of grade I embryos is the better at predicting for successful pregnancy outcome.(higher test result give more positive test).

Keywords: oocytes , pronucleus ,grade I embryo , intracytoplasmic sperm injection.

الجنين الصنف الأول هو أهم المعايير التي يمكنها التنبؤ بنتيجة الحقن المجهري

الخلاصة

كان الهدف من هذه الدراسة تقييم العوامل المؤثرة على الحقن المجهري وتحديد العامل الاهم الذي يتنبأ بنتيجة هذا الحقن.

تضمنت الدراسة خمسة وسبعين من النساء المصابات بالعقم الذين تتراوح أعمارهم بين ٢٢-٤٥ عاما (٣١.٤٣ ± ٥.٣٨ عاما) والتي تم ارسالهن إلى عيادة متخصصة للخصوبة في مدينة الصدر في النجف الاشرف لاجل الحقن المجهري ، وامتدت الدراسة طوال الفترة من مارس ٢٠١٣ إلى يناير عام ٢٠١٤ ، تم حساب معدل الإخصاب (FR) ومعدل الانقسام. تم تقييم الأجنة وفقا للشكل والنسبة المئوية للتجزئة. و تأكيد الحمل اجري في اليوم الرابع عشر من نقل الأجنة عن طريق قياس هرمون الحمل في مصل الدم .

كانت نسبة الحمل في الدراسه بعد الحقن المجهري (٢٠٪) . كان إجمالي البويضات المسحوبه من النساء ، مرحلة اخصاب البويضة و مرحلة الانقسام للبويضة و عدد الاجنه من الصنف الأول، والعدد الكلي للأجنة، والعدد الكلي للأجنة المسترجعة أعلى بكثير لدى النساء الحوامل بالمقارنة مع النساء غير الحوامل (P <٠.٠٥). تحليل منحنى ROC للتنبؤ بالحمل, حيث ان المساحة تحت المنحنى لعدد الاجنه من الصنف الأول كان ٠.٨٦٨ تليها عدد البيوض الناضجة (٠.٨١٥)، وعدد من إجمالي البيوض المسحوبه وعدد البيوض المخصبة (٠.٧٧٣)، وعدد الاجنة المسترجعة (٠.٧٦٧) وعدد الأجنة الكلي (٠.٧٥٨) على التوالي.

تشير البيانات إلى أن عدد الأجنة من الصنف الأول هو الأفضل في التنبؤ عن نتائج الحمل الناجحة.

الكلمات الرئيسية : البويضات، البيوض المخصبة، الصنف الاول للاجنه.

Introduction

The assisted reproduction methods have made a major progress and have become an integral part of the reproductive medicine. Today, the assisted reproduction techniques represent the most effective approach to the treatment of infertility[1]. They indicated for specific conditions that cause infertility, such as tubal infertility , male factor infertility or endometriosis [2 ,3]. Assisted reproductive technique (ART) involves the handling of both sperm and oocyte outside the body, to start a pregnancy [4].

Clinical situations in which ART may be useful include cases of male infertility, cases where problems with number and/or function; in addition, cases of surgically retrieval of sperm such as by sperm aspiration, in which in vitro fertilization (IVF) and ICSI may be the best approach to care [2].

Intracytoplasmic sperm injection (ICSI); involves individual sperm cell injection into oocytes cytoplasm [4]. Intracytoplasmic sperm injection is a useful technique for couples that have low or absent fertilization during IVF, or where the sperm quality and count is too low for normal IVF to be successful [5]. Assisted reproductive technique (ART) are new technologies; therefore, predictions about the success rate are less certain than other medical procedures [6]. Embryos transfer are done mostly on day 2 or 3 after fertilization in order to select the most appropriate ones for transfer [7].

Patients and Methods

This prospective cohort study was approved by approved by the Ethics Committee of the University of Kufa and informed consent was obtained from all participants. A total of 75 patients were enrolled in the study from March 2013 to January 2014. All of them were recruited according to the following criteria: Non-smokers ;Free from hepatitis and HIV(by screening test); absence of any metabolic or endocrine system-associated diseases, or any other associated condition which could alter the level of free radicals like malignancy and antioxidant therapy. A human chorionic gonadotrophine (HCG) injection was given to trigger the final stages of oocyte maturation and ultrasound-guided oocyte pick-up was performed 34–36 h later. Fertilization and cleavage was assessed daily and fertilization rate (FR) and cleavage rate (CR) was calculated according to following equation respectively.

FR= (NO. of 2PN on day one / Whole NO. of oocytes injected) ×100

CR = (NO. of cleaved embryos on day/ Total NO. of 2PN)×100.

The embryos were classified according to their morphology and percentage of fragmentation.

The biochemical pregnancies was confirm on the fourteenth day of embryo transfer by measurement of serum B- HCG .The pregnancy rate (PR) is calculated by following equation:

$$PR = \frac{\text{NO. of pregnant ladies after ICSI}}{\text{Total NO. of patients who underwent ICSI}} \times 100\%$$

Statistical Analysis

Statistical analysis was performed in this study using SPSS (Statistical Package for Social Science; Version 17) program independent t-test was used to estimate differences between groups in continuous variables .The binary logistic regression analysis used to determine the odds ratio for pregnancy as dependant variable. Receiver operating characteristic (ROC) curves were generated to investigate the predictability of ICSI parameters for pregnancy. The sensitivity, specificity were calculated for the optimal cut-off levels determined by ROC curve analysis. Results are reported as (mean ±SD). $P<0.05$, was considered statistically significant [8].

Results

pregnancy rate : The pregnancy rate in studied group was 20% (N=15) as shown in figures 1 .

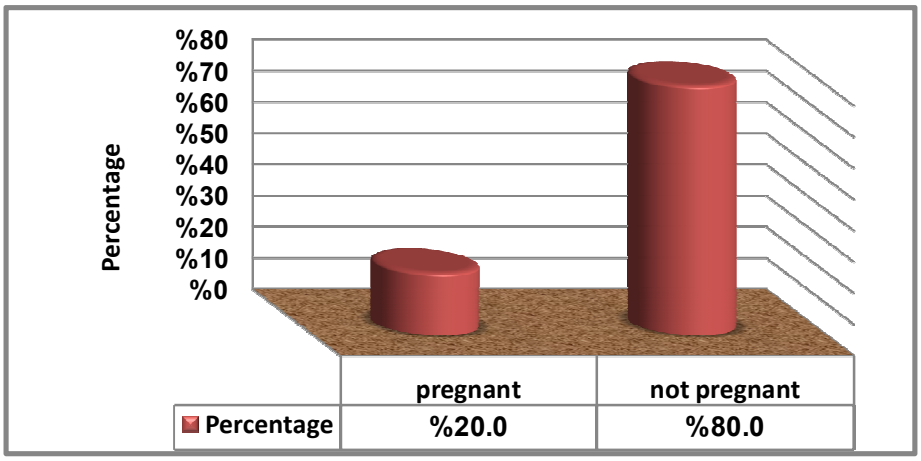


Figure (1): Pregnancy rate in studied group

Demographic Characteristics in pregnant and non-pregnant women

The Demographic Characteristics of pregnant and non-pregnant women are summarized in table (3) . Insignificant difference was noted between pregnant and non-pregnant women in terms of the woman's age, BMI, duration of infertility, occupation, residence, education and history of previous ART($P > 0.05$).

Table (3): Demographic Characteristics of patients who achieved pregnancy compared to those who did not.

Demographic Characteristics		Pregnant	Non-Pregnant	P Value
Age(Years) Mean ± SD		30.43 ± 5.00	32.18 ± 5.45	0.298
BMI(Kg/M2) Mean ± SD		27.99 ± 4.48	27.50 ± 5.53	0.775
Duration Of Infertility Years		8.54 ± 4.27	8.62 ± 4.13	0.952
Occupation	Doctor	0	1	0.861

	Engineer	0	2	
	Teacher	2	12	
	Employer	2	6	
	Student	0	2	
	Housewife	11	37	
Residence	Urban	10	39	0.903
	Rural	5	21	
Education	Illiteracy	2	8	0.334
	Primary Education	10	23	
	Secondary Education	2	9	
	High Education	2	19	
Type of Infertility	Primary	14	43	0.098
	Secondary	1	17	
PREVIOUS ART	INTRAUTERINE INSEMINATION (IUI)	1	6	0.128
	IUI & ICSI	2	20	
	Intracytoplasmic sperm injection (ICSI)	2	14	
	No	10	20	
Causes	Female Factor	8 (19.51%)	33 (80.49%)	0.703
	Male Factor	7 (25%)	21 (75%)	
	Unexplained Factor	0 (0%)	6 (100%)	
	Total	15 (20%)	60(80%)	

ICSI Parameters in pregnant and non-pregnant women

Total oocyte retrieved, stage metaphase II oocyte, pronucleus, cleavage rate , grade I, total number of embryos ,and number of transferred embryo significantly higher in pregnant women when compared with non-pregnant women($P<0.05$), while fertilization rate, grade II grade III and grade IV in pregnant women show insignificant difference when compared with non-pregnant women ($P > 0.05$) as shown in table (4).

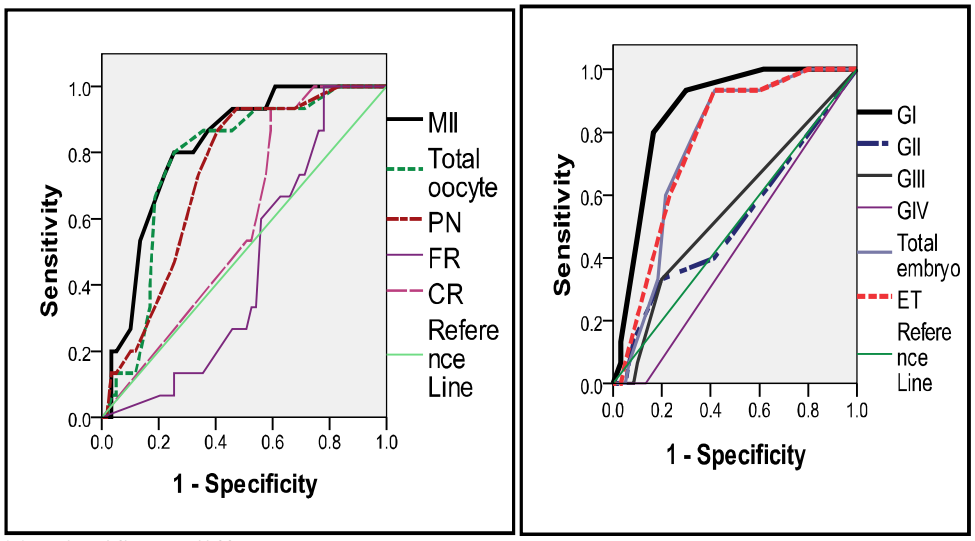
Table 4: ICSI outcome in pregnant and non-pregnant women.

ICSI Characteristics		**Pregnant**		**Non-pregnant**		**P value**	**Odds Ratio**
		Median	**Sum**	**Median**	**Sum**		
No. of retrieved oocytes	**GV**	0	16	1	65	0.567	1.08
	MI	0	14	1	73	0.340	0.85
	MII	11	160	5	364	**0.001****	1.22
	Total	12	190	7	502	**0.002****	1. 13
Pronucleus		5	84	2	202	**0.013***	1.22
Fertilization rate(%) (Mean ± SD)		53.43 ± 20.53		54.85 ± 34.33		0.838	0.87
Cleavage rate % (Mean ± SD)		87.65 ± 15.60		68.63 ± 39.64		**0.005****	1.77

Embryo grading	**Grade I**	3	51	1	75	**0.001****	2.27
	Grade II	0	13	0	47	0. 817	1.06
	Grade III	0	6	0	29	0.380	0.93
	Grade IV	0	0	0	11	0.138	-
Total embryo		5	70	2	162	**0.002****	1.41
Number of transferred embryo		4	53	2	129	**0.001****	1.81

* significant differences at P<0.05
** significant differences at P<0.01
MI metaphase I , MII metaphase II, GV ,germinal vesicle

In binary logistic regression analysis, number of oocyte retrieved, metaphase MII, Pronucleus, grade I embryo and total number of embryo were significantly associated with the positive pregnancy. Increased number of oocyte retrieved , metaphase MII, Pronucleus, grade I embryo, number of total embryo were associated with increased odds ratio for the positive pregnancy (OR = 1.13 , [1.021-1.240] ; OR = 1.22, [1.096-1.388] ; OR = 1.22, [1.015-1.462] ; OR = 2.27, [1.475-3.503] ; OR = 1.41, [1.092-1.823] and OR = 1.81 , [1.174-2.795] respectively (Table 4.4). All pregnant women do not have grade IV embryo, So no odds ratio was mentioned for grade IV embryos. The sensitivity and specificity of the ICSI parameters for positive pregnancy and the best cutoff point associated with pregnancy are shown in figure-2. Analysis of ROC curve revealed that area under the curve for number of grade I embryos was 0.868 for predicting pregnancy followed by followed by MII (0.815), number of total oocyte .retrieved and PN (0.773), ET (0.767) and total number of embryos (0.758) respectively.



** significant differences at P<0.01

Variables	AUC	Cut-off Point	Sensitivity	Specificity	95% CI	
					Lower Bound	Upper Bound
MII	.815	9	77	76	.710	.919
Oocyte retrieval	.773	10	73	78	.652	.893
PN	.733	3	90	56	.611	.856
FR	.454	52	44	52	.317	.591
CR	.589	68	93	41	.451	.728
Grade I	.868	2	87	77	.780	.952
Grade II	.517	2	23	86	.350	.695
Grade III	.554	1	20	85	.397	.729
Grade IV	.433	0	0	91	.281	.584
TE	.758	3	87	63	.657	.884
ET	.767	3	77	68	.666	.893

Figure (2): Estimated area under curve (AUC) and predictive cut-off point values for the individual intracytoplasmic sperm injection parameters with the receiver operating characteristics (ROC) curve analysis between pregnant and non-pregnant women.

Discussion

Pregnancy rate

In the present work, the pregnancy rate in the studied group was 20% (N=15) as shown in figures 2. Ferraretti *et al.* mentioned that the pregnancy rates for ICSI in 21 European countries, was 28.7 % [9]. Pregnancy rate in this fertility center slightly lower than ICSI centers in European countries this may be due to their progression in this field , presence of facilities like instruments , types of culture media and their experience.

Demographic Characteristics in pregnant and non pregnant women

The Demographic Characteristics of pregnant and non pregnant women are summarized in table (3), No significant difference was noted between pregnant and non-pregnant group in terms of the woman's age, BMI, duration of infertility, occupation, residence, education and previous ART ($P > 0.05$).This result agrees with Yalçınkaya et al.'s and Usoniene et al.'s results [10, 11].

About maternal age, Ashrafi et al. found that there was significant deference between two groups ($p<0.05$) [25]. Also, Ashrafi, *et al.* found that there was insignificant difference noted between pregnant and non-pregnant women in terms of the BMI, type of infertility and number of previous trial for ART [12].Other studies indicated that BMI was significantly higher in non pregnant women when compared to pregnant women [26]. No significant difference was noted between pregnant and non-pregnant women in terms of cause and duration of infertility. These results agree with Yuan, et al and Bedaiwy et al. [13,

14]. This might be related to our study's strict inclusion criteria or may be due to the number of patients.

ICSI parameters in pregnant and non pregnant women

Total oocyte retrieved, stage MII oocyte, PN, CR grade I, the total number of embryos, and number of ET were significantly higher in pregnant women when compared with non-pregnant women ($P<0.05$), while FR, grade II grade III and grade IV in pregnant women show insignificant difference when compared with non-pregnant women ($P > 0.05$) as shown in table (4.4).These results agreed with van Loendersloot et al.'s and Berger, et al. results [15, 16].

Van Loendersloot et al. found a direct relationship between increasing number of oocytes retrieved, ET, embryo quality and pregnancy rate after ART [17]. Berger, et al. found that pregnancy rate was significantly associated with number of mature oocytes retrieved, number of embryos at day 3and grade of embryos transferred on day 3 ($p < 0.05$) [18].

In contrast Gowda, et al. were not able to demonstrate any association between fertilization potential of oocytes and IVF outcome [19]. These results were more confirmed by binary logistic regression analysis. See table (5). In binary logistic regression analysis, number of oocyte retrieved, MII, PN, grade I embryo and the total number of embryo were significantly associated with the positive pregnancy. The increased number of oocyte retrieved, MII, PN, grade I embryo, the number of total embryo were associated with increased odds ratio for the positive pregnancy (OR = 1.13 , [1.021-1.240] ; OR = 1.22, [1.096-1.388] ; OR = 1.22, [1.015-1.462] ; OR = 2.27, [1.475-3.503] ; OR = 1.41, [1.092-1.823] and OR = 1.81, [1.174-2.795] respectively (Table-4). All pregnant women do not have grade IV embryo and so no odds ratio was mentioned for grade IV embryos.

Our data confirms previous data of Racowsky et al.; Vernon, et al., and Stephanie et al. who reported that embryo grading significantly associated with and predicted pregnancy after ICSI [20 ,21 and 22]. Van Loendersloot et al. found that pregnancy chances had significant association with ICSI parameters, with a summary OR of 1.04 (95% CI: 1.02–1.07) [29].

The sensitivity and specificity of the ICSI parameters for positive pregnancy and the best cutoff point associated with pregnancy are shown in figure (4.2). Analysis of ROC curve revealed that area under the curve for number of grade I embryos was 0.868 for predicting pregnancy followed by MII (0.815), number of total oocyte. retrieved and PN (0.773), ET (0.767) and total number of embryos (0.758) respectively. These results were in agreement with Weitzman, et al., Cai, et al., and Roberts et al. results.

Weitzman, et al. found that embryo grading are helpful in the prediction of implantation rate, particularly, embryo grade and cell number are more important factors for selecting proper embryos for transfer [22].

Cai et al. found that the total number of grade I and II embryos was a superior predictor for ART success [23]. Cai, et al. also establish that embryonic parameters, including total number of grade I & II embryos and total number of embryos, ordered as the first most important and second most important predictors for ART success [23]. These results recommend that the total number of embryos give additional information to predict ART success if there was lack in determination of the total number of good-quality embryos. It may be a substitution marker for hormonal factors and may work through the uterine receptivity [24].

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